Program/Abstract # 533
Snail2–PHD12 interaction recruits an epigenetic repressive complex that mediates neural crest epithelial–mesenchymal transition
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The epithelial to mesenchymal transition (EMT) is a process by which epithelial cells are converted from a tightly adherent sheet of cells into a more dispersed mesenchymal population. Neural Crest (NC) cells are a transient pluripotent cell population that delaminate from the dorsal neural tube via an EMT, migrate extensively and contribute to numerous derivatives. It is well-known that Snail represses cadherin expression, guiding changes in the adhesion properties prior NC EMT. However, the molecular mechanisms underlying its repressive activity were unknown. Here, we characterize a Plant-Homeodomain factor (PHD12) that we show interacts with both Snail2 and the Sin3A/HDAC repressive complex using an in vivo immunoprecipitation assay. PHD12 knock-down embryos were used for a multiplex Nanostring expression analysis, evidencing an elevated Cadherin6b (Cad6b) and low NC specifier makers (Sox10, FoxD3, Snail2) in the dorsal neural tube after loss of PHD12. These results were validated by immunohistochemistry using specific antibodies showing lack of migrating NC together with an increase in Cad6b expression at the dorsal aspect of the neural tube. Using an in vivo chromatin immunoprecipitation (ChIP) assay we provide evidence that PHD12 protein interacts with the Cad6b locus only prior NC migration, concomitantly with promoter hypoacetylation. Taken together, we provide the first in vivo evidence showing the molecular mechanism of Snail2 repression via association with an epigenetic repressive complex that deacetylates the promoter of a target gene, Cad6b.

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Program/Abstract # 534
LMO4 modulates slug/snail function in neural crest development
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The neural crest (NC) is a population of multipotent stem cell-like precursors that arise at the neural plate border in vertebrate embryos. These cells undergo an epithelial to mesenchymal transition (EMT) and migrate extensively before giving rise to a diverse set of derivatives. Interestingly, components of the neural crest gene regulatory network (NC-GRN) are often used reiteratively to control multiple steps in the development of these cells. For example, Slug and Snail are required for the formation of the NCs as well as for their subsequent EMT/migration. It is important to understand the mechanisms that control the distinct function of these factors in different cellular contexts. For example, Snail family transcription factors may assemble different co-regulatory complexes in different enhancer contexts, and it is therefore essential to characterize their interaction partners, particularly those that might play a scaffolding role. Here we show that the LIM adaptor protein, LMO4, is a Slug/Snail interacting protein that is essential for NC development. LMO4 is expressed in NC forming regions of the embryo, as well as in the central nervous system and the cranial placodes. LMO4 binds directly to Slug and Snail, but not to other components of the NC-GRN. In addition, LMO4 can modulate Slug-mediated neural crest induction, suggesting a mechanistic link between these factors. LMO4 is necessary for normal NC development as morpholino-mediated "knockdown" of this factor leads to loss of NC precursor formation at the neural plate border. Importantly, misexpression of LMO4 leads to ectopic expression of some neural crest markers but a reduction in the expression of others. This suggests that a better understanding of the function of LMO4 in NC development will help delineate the complex regulatory relationships between different components of the NC-GRN.

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Program/Abstract # 535
Regulating the function of Twist, an essential factor in neural crest development and tumor progression
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Neural crest cells are multipotent, proliferative stem cells that undergo an epithelial–mesenchymal transition (EMT) and migrate to distant regions of the embryo where they give rise to a large and diverse set of derivatives essential to the vertebrate body plan. Interestingly, a number of transcription factors that control EMTs during neural crest development, including Twist, Snail, Slug, and Sip1, also regulate tumor cell metastasis. Twist is a bHLH (basic helix–loop–helix) protein expressed during neural crest formation, migration, and fate diversification. Depletion of Twist in Xenopus leads to defects in cranial neural crest formation and migration, demonstrating its necessity for the normal development of these cells. The mechanisms via which Twist regulates cell fate decisions, as well as EMTs in both embryonic development and cancer metastasis, remain poorly understood. Here we show that an E3 ubiquitin ligase, Ppa, that had previously been shown to regulate Slug/Snail protein levels, also regulates Twist. Ppa binds Twists highly conserved WR domain and promotes its instability. Our findings suggest that the ubiquitin–proteasome system modulates the protein expression levels of multiple structurally distinct EMT regulatory factors, including Twist, Snail, Slug, and Sip1, through the actions of a common E3 ligase. The WR domain can mediate additional protein–protein interactions that impact Twist function during neural crest development, distinct from its effect on Twist stability. Together our findings provide novel insights into the function of a key regulator of both early embryonic development and tumor progression.

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Program/Abstract # 536
Twist1 directly regulates genes associated with cell proliferation and migration in developing heart valves
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Congenital heart defects, including valve malformations, are among the most common birth defects in the world. Heart valves develop from extracellular matrix (ECM) rich endocardial cushions (ECCs) populated by highly proliferative, migratory, and undifferentiated mesenchymal cells. The basic helix–loop–helix transcription factor Twist1 promotes cell proliferation and migration in cultured ECC mesenchymal cells. Although implicated as a regulator of essential mesenchymal functions, Twist1 direct transcriptional targets remain largely unknown. We hypothesize that Twist1 directly regulates transcription of genes promoting cell proliferation and migration in ECC mesenchymal cells. Two candidate genes, Tbx20 and Cadherin-11 (Cdh11), contain evolutionarily conserved regions (ECRs) with putative Twist1 binding E-box consensus sites. ECRs

associated with Tbx20 and Cdh11 are transactivated by Twist1 in an E-box dependent manner and are directly bound by Twist1 in mouse ECC mesenchymal cells. Additional Twist1 candidate genes were identified through Affymetrix microarray analysis of MC3T3-E1 cells treated with control siRNA or Twist1 siRNA. Loss of Twist1 resulted in decreased expression of genes associated with cell proliferation, migration, and ECM components. E-box consensus sites were identified within ECRs of Semaphorin3C, Gadd45a, and Rab39b. All three ECRs are directly bound by Twist1 in mouse ECC mesenchymal cells. These studies identified Twist1 direct transcriptional targets promoting cell proliferation and migration in ECC mesenchymal cells. Therefore, these Twist1 transcriptional target genes likely contribute to maintenance and expansion of valve progenitor cells during early stages of valve development.

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Program/Abstract # 537
Co-regulation of mutual target genes by Ntla and Tbx16 in zebrafish mesoderm development
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T-box genes (e.g. Ntla and Tbx16) encode transcription factors that govern critical aspects of early embryonic development. We have performed promoter ChIP-on-chip microarrays for Ntla (Morley et al 2009) and Tbx16. Based on these data, we extracted 35 targets that were potentially co-regulated by Ntla and Tbx16, based on co-occupation of upstream genomic regions. We performed an in situ hybridization screen using ntla, spt (Tbx16 mutant) and ntl; spt mutant embryos. A subset of these targets showed mutual regulation by Ntla and Tbx16 and we confirmed these observations using ntl and/or Tbx16 morpholinos. Subsequently, we tested this subset for direct regulation by Tbx16 and Ntla by protein synthesis inhibition. We confirmed binding of Ntla and Tbx16 to the genomic regions identified by ChIP in EMSA and yeast 1-hybrid assays. To assay for function we used luciferase and mCherry reporter assays in vivo. Here we will present the data for each individual target tested. We used this information and previously published data to build a GRN regulatory demarcating overlapping and non-overlapping targets of Tbx16 and Ntla in different aspects of mesoderm development.

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Program/Abstract # 538
Multiple mechanisms negatively regulate *C. elegans* tbx-2 expression
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The *C. elegans* T-box gene tbx-2 is necessary for pharyngeal muscle development and differentiation of a subset of neurons. We are interested in understanding how tbx-2 is regulated. In wild-type animals, a tbx-2::gfp reporter is expressed in pharyngeal precursors and in several neurons. Using an RNAi-based screen to identify transcription factors regulating tbx-2 expression, we have identified the NF-Y complex and TBX-2 itself as negative regulators of tbx-2. NF-Y is a heterotrimeric CCAAT-binding complex consisting of A, B and C subunits, and reduction of the NF-Y subunits nfya-1, nfyb-1, or nfyc-1 by RNAi or using mutants results in ectopic tbx-2::gfp expression in hypodermal seam cells and gut. Mutation of two CCAAT sites in the tbx-2 promoter results in a similar pattern of ectopic tbx-2::gfp expression, suggesting that NF-Y directly represses the tbx-2 promoter. Reduction of tbx-2 by RNAi or using a hypomorphic tbx-2 mutant also results in ectopic tbx-2::gfp expression in seam cells and gut. While tbx-2 expression has not been previously observed in these tissues, recent whole genome RNA profiling studies suggest tbx-2 is broadly expressed. We find that mutation of a T-box factor binding site in the tbx-2 promoter results in ectopic tbx-2::gfp expression, consistent with direct autoregulation. We have previously suggested that TBX-2 is a SUMOylation-dependent transcriptional repressor, and we find that RNAi of SUMOylation components results in ectopic tbx-2::gfp expression in seam cells and gut, and in additional tissues. We suggest the tbx-2 promoter is directly repressed by TBX-2 and perhaps other SUMOylation dependent transcription factors.

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Program/Abstract # 539
Examining the role of SUMOylation in *C. elegans* T-box transcription factor TBX-2 function
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T-box transcription factors are key developmental regulators, but despite their importance, little is known about how these proteins are regulated. Based on a previous work, we hypothesize the *C. elegans* T-box factor TBX-2 functions as a SUMO-dependent transcriptional repressor. Here we demonstrate TBX-2 function depends on SUMOylation *in vivo*. Further, we show that TBX-2 interaction with SUMOylation enzymes is mediated by two consensus SUMOylation sites located in the T-box DNA-binding domain and near the TBX-2 C-terminus, respectively. TBX-2 is SUMOylated upon co-transfection with human SUMO-1 protein in COS-1 cells. Mutation of the SUMO-attachment lysine within the C-terminal SUMO consensus site has a significant effect on total TBX-2 SUMOylation, whereas mutation of the T-box site lysine has a more modest effect on SUMOylation. In comparison, a more severe mutation converting these SUMO consensus sites to all alanines completely eliminated SUMOylation. To examine the effect of SUMOylation on TBX-2 function, we compared wild-type TBX-2 to the mutant in which the SUMO-consensus sites were mutated to alanines. SUMOylation is not necessary for normal TBX-2 nuclear localization in either *C. elegans* or COS-1 cells. Likewise, while wild-type TBX-2 fused to the Gal4 DNA-binding domain can repress a Gal4 binding-site containing reporter, SUMOylation is not necessary for this repression. We suggest that SUMOylation may affect TBX-2 DNA-binding or interaction with other factors bound to target promoters. We are currently developing an assay to determine if TBX-2 is a SUMO-dependent repressor using a TBX-2 regulated promoter in *C. elegans*.

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Program/Abstract # 540
***C. elegans* TBX-2 is a SUMOylation dependent transcriptional repressor**
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T-box transcription factors are crucial developmental regulators in all multicellular animals, and they have been implicated in a variety of human diseases and cancers. Despite their importance, few direct targets of T-box factors have been identified. *C. elegans* TBX-2 is a